
The nucleotide sequence of *Euglena* cytoplasmic phenylalanine transfer RNA. Evidence for possible classification of *Euglena* among the animal rather than the plant kingdom

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ABSTRACT

The nucleotide sequence of cytoplasmic phenylalanine tRNA from *Euglena gracilis* has been elucidated using procedures described previously for the corresponding chloroplastic tRNA [Cell, 9, 717 (1976)]. The sequence is: pG-C-C-G-A-C-U-U-A-m²G-C-U-Cm-A-G-D-D-G-G-G-A-G-A-G-C-m²G-ψ-ψ-A-G-A-Cm-U-Gm-A-A-Y-A-ψ-C-U-A-A-A-G-m⁷G-U-C-*C-C-U-G-G-T-ψ-C-G-m¹A-U-C-C-C-G-G-G-A-G-ψ-C-G-G-C-A-C-C-A. Like other tRNA^{Phe}s thus far sequenced, this tRNA has a chain length of 76 nucleotides. The sequence of *E. gracilis* cytoplasmic tRNA^{Phe} is quite different (27 nucleotides out of 76 different) from that of the corresponding chloroplastic tRNA but is surprisingly similar (72 out of 76 nucleotides identical) to that of tRNA^{Phe} from mammalian cytoplasm. This extent of sequence homology even exceeds that found between *E. gracilis* and wheat germ cytoplasmic tRNA^{Phe}. These findings raise interesting questions on the evolution of tRNAs and the taxonomy of *Euglena*.

INTRODUCTION

The origin of mitochondria and chloroplasts is the subject of much controversy. Perhaps the most widely accepted theory to explain their origin is the endosymbiotic hypothesis (1-3). However, an alternative hypothesis suggests that these organelles may have arisen internally from different genomes within an ancestral eukaryotic cell (4,5).

Previously we have reported the nucleotide sequence of the chloroplastic tRNA^{Phe} from *Euglena* (6-8). A comparison of the sequence of this tRNA with those of other tRNA^{Phe}s (three from prokaryotes and three from eukaryotic cytoplasm) showed that both in the nature of its modified nucleosides and in its sequence chloroplastic tRNA^{Phe} appears to be more homologous with prokaryotic tRNA^{Phe}s (although all tRNA^{Phe} sequences show great homology) (9). These observations indicate that perhaps *Euglena* chloroplasts and extant prokaryotes shared a common ancestor. Such interpretations might be enhanced by comparing the nucleotide sequences of chloroplastic and cytoplasmic tRNA^{Phe}s from the same organism. In this paper we therefore present the nucleotide sequence of *Euglena* cytoplasmic

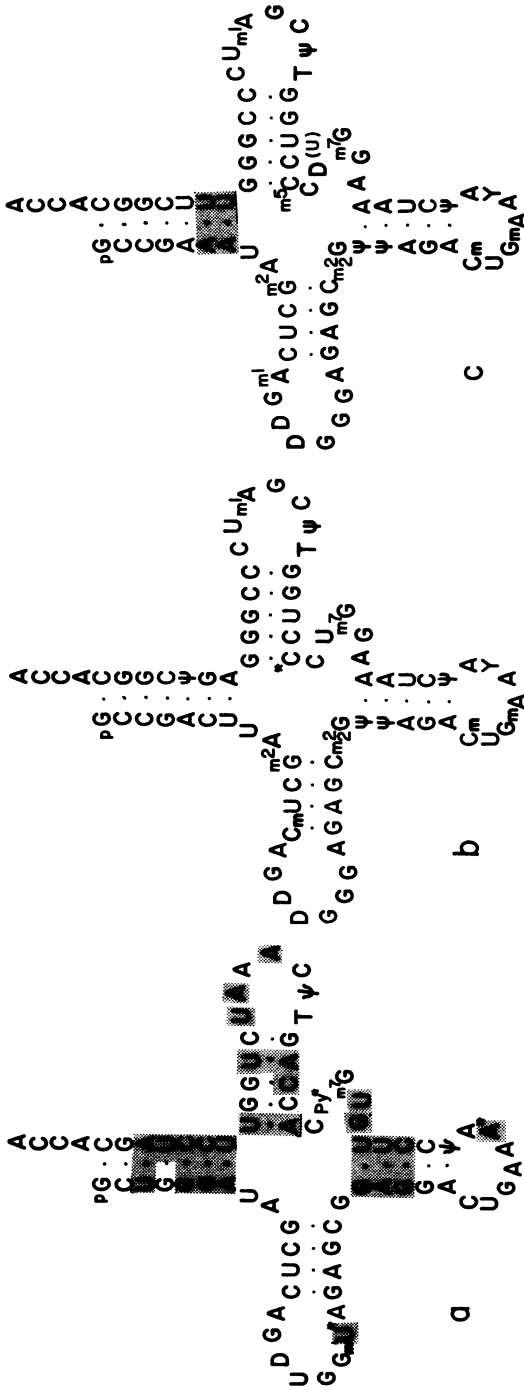


Figure 2. Cloverleaf models of Euglena cytoplasmic tRNA^{Phe} (b) *Euglena* chloroplastic tRNA^{Phe} (a) and beef liver tRNA^{Phe} (c). Hatched areas indicate sequence differences when *Euglena* cytoplasmic tRNA^{Phe} is compared with the corresponding chloroplastic tRNA^{Phe} (a and b) and beef liver tRNA^{Phe} (b and c).

graphic patterns for the corresponding fragments during our earlier work.

For the purposes of comparison, the cloverleaf structures of Euglena chloroplastic tRNA^{Phe} (Fig. 2a) and that of mammalian cytoplasmic tRNA^{Phe} (Fig. 2c) are also shown in the same figure. Like 17 other tRNA^{Phe}s thus far sequenced, Euglena cytoplasmic tRNA^{Phe} also has a chain length of 76 nucleotides. Based on the modified nucleosides present in this tRNA and their location within the tRNA and its overall sequence homologies with other tRNA^{Phe}s (9) Euglena cytoplasmic tRNA^{Phe} is a typical eukaryotic tRNA. Thus it contains m¹A, m⁵C, m²G, m²G, and Y, modified nucleosides which are characteristic of eukaryotic tRNA^{Phe}s.

DISCUSSION

Besides the finding that the general features of Euglena cytoplasmic tRNA^{Phe} are more similar to those of eukaryotic cytoplasmic rather than prokaryotic tRNA^{Phe}s, the most surprising result of this work is that the sequence of Euglena cytoplasmic tRNA^{Phe} is virtually identical to that of mammalian cytoplasmic tRNA^{Phe} (15-17) (Fig. 2b and c) and differs from it in only 4 out of 76 nucleotides. This extent of sequence homology (94.7%) is significantly higher than the homology of Euglena cytoplasmic tRNA^{Phe} to the corresponding tRNA from wheat germ or pea seedling (78.9%), baker's yeast (80.3%) and Schizosaccharomyces pombe cytoplasm (67.1%) (18). This result raises an interesting question about the taxonomy of Euglena, which because of their "plant-like" or "animal-like" characteristics has long been an enigma to biologists. The evidence presented here indicates that Euglena would more appropriately be classified as an animal rather than as a plant. It would clearly be of interest to investigate this further and examine whether this sequence homology between Euglena and mammalian cytoplasmic tRNA^{Phe} extends to other tRNAs as well.

The sequence of Euglena cytoplasmic tRNA^{Phe} is quite different from that of the corresponding chloroplastic tRNA. As shown in Figures 2a and 2b, these two tRNAs differ in 27 out of 76 nucleotides. This finding is in accord with the hypothesis that the chloroplastic genome and nuclear genome may have evolved from different ancestors (1-3). A similar difference between bean chloroplastic tRNA^{Phe} and bean leaf cytoplasmic tRNA^{Phe} has also been described (19).

In contrast to the substantial sequence differences between the chloroplastic and the corresponding cytoplasmic tRNA^{Phe}s, the two chloroplastic tRNA^{Phe}s that have been sequenced have virtually identical sequences

TABLE I. Sequence Homology between Various Phenylalanine tRNA (%)^a

Source of tRNA	B.sub- tilis	E. coli	A. ni- dulans	Bean Chl.	Eugl. Chl.	Yeast mito.	Yeast cyto.	S. pombe	Wheat germ	Mamm. Mamm.	Eugl. cyto
Mycoplasma	82.9	72.4	78.9	69.7	68.4	57.8	63.2	55.3	64.5	61.8	61.8
B. subtilis		75.8	81.6	76.3	71.8	56.6	60.5	64.5	65.8	63.2	64.5
E. coli			84.2	78.9	70.0	63.1	63.2	56.6	64.6	71.0	68.4
A. nidulans				86.8	82.9	55.3	59.2	59.2	69.7	69.7	67.1
Bean Chl.					<u>93.4</u>	60.5	59.2	59.2	67.1	67.1	64.5
Eugl. Chl.						63.1	61.8	61.8	69.7	67.1	<u>64.5</u>
Yeast mito.							61.8	56.9	61.8	60.5	<u>59.2</u>
Yeast cyto.								64.5	84.2	77.6	80.3
S. pombe									64.5	72.4	67.1
Wheat germ										81.6	78.9
Mammal											<u>94.7</u>

^a. Comparison of the number of identical parental nucleotides between two different tRNA^{Phe}s.

(Table I), 93.4% homology (19). Furthermore, the two chloroplastic tRNAs are generally more homologous to prokaryotic tRNA^{Phe}s (68.4-86.8%) than to eukaryotic tRNA^{Phe} (59.2-69.7%) and within the prokaryotes more to blue green algae tRNA^{Phe} (Chang *et al.*, unpublished results) than to the corresponding tRNA from mycoplasma, *B. subtilis* or *E. coli*. These results are in accord with the hypothesis that chloroplasts evolved from a common prokaryotic endosymbiont, which is more likely similar to current day blue green algae. Furthermore, analysis of oligonucleotides present in T1 RNase digests of red algae chloroplastic 16s rRNA (20) and a more recent paper on sequence of 3'-terminal of maize chloroplastic 16s rRNA (21) also provide independent evidence for the prokaryotic origin of chloroplasts.

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